

PATENT SPECIFICATION

(11) 1 395 330

(21) Application No. 59296/71 (22) Filed 21 Dec. 1971

(23) Complete Specification filed 20 Dec. 1972

(44) Complete Specification published 21 May 1975

(51) INT CL²: C11D 3/386; C07G 7/02

(52) Index at acceptance

C5D 6A5C 6A5D2 6B12N1 6B1 6B2 6B3 6B8 6C6 6D
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(54) ENZYMIC DETERGENT COMPOSITIONS

(71) We, UNILEVER LIMITED, a company organised under the laws of Great Britain, of Unilever House, Blackfriars, London, E/C 4, England, do hereby declare the invention for which we pray that a patent may be granted to us and the method by which it is to be performed, to be particularly described in and by the following statement:—

The present invention relates to enzyme-containing granules and granular detergent compositions incorporating enzyme-containing granules.

Enzyme-containing granules are well-known in the art. They have been particularly developed for use in granular detergent compositions. They are commonly prepared by granulating powdered enzyme products with the aid of a granulating agent, or by affixing powdered enzyme products onto a carrier material with the aid of a conglutinating agent. Another method is to affix powdered enzymes onto a hydratable carrier material with the aid of water, either by spraying an amount of water onto a mixture of powdered enzymes, or by spraying an aqueous solution of the enzymes onto the hydratable carrier material. The above methods for preparation of enzyme-containing granules have e.g. been described in Dutch patent application 6915293, which has been laid open to public inspection, and UK patent specifications 1156238 and 1151748.

In comparison with powdered enzyme preparations, enzyme-containing granules are more suitable for inclusion in granular detergent compositions in that they are more stable therein than powdered enzyme preparations.

However, if the enzymes in such enzyme-containing granules are amylolytic enzymes, the storage stability of such amylolytic enzyme-containing granules is often not satisfactory.

It has now been found that the storage stability of amylolytic enzyme-containing granules can be significantly improved by inclusion in those granules of an amino acid, preferably an α -amino acid.

It has been proposed in the art to use amino acids to stabilize particular proteolytic

enzymes in solution. Thus, for example, U.S. patent specification 3,051,627 describes the use of amino acids to stabilize α -chymotrypsin in aqueous solution, and German patent specification 1,185,768 discloses the use of amino acids to stabilize aqueous trypsin solutions. This prior art is however concerned with the stabilization of proteolytic enzymes in aqueous solutions.

It has furthermore been disclosed in U.S. patent specification 3,560,392 to stabilize proteolytic enzymes in granules by inclusion therein of a partially hydrolysed and partially solubilized collagen as stabilizer for said proteolytic enzymes. This art however is concerned with the stabilisation of proteolytic enzymes by means of a specific substrate protection. This necessarily implies that completely hydrolysed proteins are not suitable, which is clearly revealed in said specification.

It has now surprisingly been found that amino acids and particularly α -amino acids are effective improvers of the storage stability of amylolytic enzyme-containing granules, in which the amylolytic enzymes are in intimate contact with the amino acid or derivatives thereof.

It is to be remarked that German Offenlegungsschrift 1,942,236 describes a particulate detergent composition which contains amino acids and, as the case may be, enzymes, including amylolytic enzymes, in granular form. The amino acids are however incorporated as such in the particulate detergent composition, and not in the enzyme-containing granules. Furthermore German patent specification 729,667 describes the inactivation of pectin-degrading enzymes, present in amylase-containing enzyme preparations by treatment of the enzyme mixture with aqueous solutions of α -amino acids. The use of α -amino acids in amylolytic enzyme-containing granules is however not revealed therein.

The present invention therefore relates to amylolytic enzyme-containing granules with improved storage stability, said granules containing a stabilizing amount of an amino acid, particularly an α -amino acid.

The amylolytic enzymes to be used in the present invention may be of vegetable or animal origin or may be derived from micro-organisms, particularly from bacteria or fungi. Suitable amylolytic enzymes of vegetable or animal origin are malt amylolytic enzymes, pancreatic amylolytic enzymes. Preferably α -amylases are used. Suitable examples of amylases from bacteria are those produced by the *Bacillus* species, in particular by strains of *B. subtilis*. These amylases are commercially available, e.g. α -amylase ex Novo Industri A/S, Copenhagen, Denmark, Maxamyl ex Royal Fermentation Industries, Delft, Holland, bacterial α -amylase ex Wallerstein Co., New York. Amylolytic enzymes from fungi are e.g. those derived from *Aspergillus oryzae*, *A. Niger*, and so on. The amylolytic enzymes, as they are commercially available, often contain minor amounts of other enzymes, such as proteases, lipases, cellulases, maltases and glucosidases.

The α -amino acids to be used in the present invention are the common amino acids from proteins. Examples of such α -amino acids are glycine, alanine, valine, leucine, isoleucine, phenylalanine, tyrosine, proline, hydroxyproline, serine, threonine, cysteine, cystine, methionine, thryptophane, aspartic acid, glutamic acid, arginine, lysine, histidine and ornithine. Completely hydrolysed proteins are also suitable in the present invention. Although all α -amino acids improve the storage stability, particularly preferred are glycine, histidine, hydroxyproline, leucine, isoleucine, alanine, glutamine, proline and serine, as well as fully hydrolyzed proteins such as fully hydrolyzed casein, wheat and soyabean meal. These proteins are completely hydrolyzed in any suitable way, e.g. with hydrochloric acid, followed by neutralizing the obtained acid hydrolysate with e.g. sodium hydroxide and filtering the resulting product. Treatment with active carbon is then applied to improve the colour of the filtered product. Especially suitable are glycine and fully hydrolyzed casein. Mixtures of various α -amino acids may of course also be used.

The amino acids, other than α -amino acids to be used in the present invention are e.g. β -alanine and γ -aminobutyric acid. Preferably α -amino acids are used in the present invention.

The amino acids are used in the amylolytic enzyme-containing granule in a stabilizing amount. In general, the weight ratio of the amino acids to the amylolytic enzymes is from

$$10 \text{ to } \frac{1}{1000}, \text{ preferably from } 1 : \frac{1}{10}.$$

The amount of amylolytic enzymes in the granule is generally 1 to 25, preferably 5 to 15% by weight.

The granules of the invention can be prepared in any suitable way, as long as the

amylolytic enzymes are brought into intimate contact with the amino acids. Thus for example granules can be made from a dry mixture of powdered amylolytic enzymes and the amino acids with the aid of a suitable granulating agent such as nonionic surface active agents. This granulation method is e.g. described in Dutch patent application 6915293, which has been laid open to public inspection.

Another suitable method is to conglutinate an intimate mixture of powdered amylolytic enzymes and amino acids onto a carrier material, in which a suitable granular carrier material, such as granular sodium tripolyphosphate is rendered glutinous by spraying on the granules a liquid or liquefied nonionic surface active agent, and subsequently the mixture of amylolytic enzymes and amino acids is added to these glutinous granules, conglutinating the latter with the powder. A modification thereof is that the mixture of amylolytic enzymes and amino acids is dispersed in the nonionic surface active agent and sprayed upon a suitable granular carrier material.

A preferred method to prepare the granules of the invention is to attach the amylolytic enzymes in the presence of the amino acids to a hydratable carrier material by the process as described in UK patent specification 1151748. Particularly preferred is sodium triphosphate as carrier material. This can be effected by making a dry mixture of the amylolytic enzymes, the carrier material and the amino acids and adding a sufficient amount of water to this mixture while agitating. Another method is to add sufficient water to the powdered amylolytic enzymes plus the amino acids to prepare a slurry like dispersion and mix this dispersion with the carrier material in a suitable mixing device.

The mixture of amylolytic enzymes with the amino acids, from which granules can be prepared, can be obtained in any suitable way. The amylolytic enzymes may be precipitated or adsorbed from the mother liquor in the presence of the amino acids, or the mother liquor containing the amylolytic enzymes and amino acids may be evaporated or spray-dried. The products thus obtained may then be granulated in a manner as described before.

Another preferred method is to freeze-dry an aqueous solution of a mixture of amylolytic enzymes with the amino acids and subsequently attach this freeze-dried mixture to a granular carrier material by the method as described in U.S. patent specification 3,519,570.

The amylolytic enzyme-containing granules of the invention are particularly useful for inclusion in a granular detergent composition, either a fabric washing composition or a dishwashing composition (for use as a (pre)soaking, main wash or rinse dishwashing composition).

The granular detergent composition for

fabric washing may be a spray dried or dry-mixed detergent composition, generally containing an organic detergent surfactant, inorganic and/or organic builder salts and further common detergent adjuvants.

The organic detergent surfactant may comprise an anionic, a nonionic synthetic detergent, a soap or mixtures thereof.

Examples of suitable anionic detergent surface-active agents are alkali-metal salts of alkylbenzene sulphonates with 12 to 18 carbon atoms in the alkyl group; of C_{10} - C_{24} alkyl sulphates and sulphonates; of C_{10} - C_{20} olefin sulphonates which are sulphonation products of C_{10} - C_{20} olefins, particularly α -olefins, which have been neutralized and hydrolyzed. Further suitable examples of anionic detergent surface-active agents can be found in Schwartz, Perry and Berch "Surface-active Agents and Detergents", Volume II, 1958 under the heading "Anionic Surfactants".

Examples of suitable nonionic detergent surface-active agents are ethylene oxide or propylene oxide condensation products with primary or secondary monohydric C_{12} - C_{24} alcohols, with C_8 - C_{18} alkylphenols, with C_{10} - C_{24} fatty acid amides, with polyalkylene glycols and mixed alkylene oxide condensation products. Further suitable examples can be found in the above-mentioned reference Schwartz, Perry and Berch under the heading "Nonionic Surfactants".

Examples of suitable soaps are the alkali-metal salts of C_{12} - C_{22} fatty acids such as coconut oil, palm oil, fish oil, and tallow fatty acids. The fatty acids may be natural or synthetic fatty acids. Further examples can be found in the reference Schwartz, Perry and Berch under the heading "Soaps".

The detergent composition furthermore comprises a water-soluble alkali-metal builder salt. This is a salt which increases the level of detergency attainable by a detergent surface-active agent. Suitable examples of water-soluble alkali-metal builder salts are alkali-metal orthophosphates, pyrophosphates, tripolyphosphates, aminopolycarboxylates like ethylenediaminetetraacetate and nitrilotriacetates, alkali-metal carbonates and silicates.

Mixtures of the above builder salts may also be used.

The detergent composition may furthermore comprise an oxygen-liberating bleaching agent. Suitable examples thereof are persalts, like alkali-metal percarbonates and perborates.

The detergent composition may furthermore comprise the normal detergent adjuvants such as lather boosters like coconut ethanolamide; soil-suspending agents like sodium carboxymethyl cellulose; hydrotropes like sodium toluene sulphonate; activators for the bleaching agent like tetraacetythylenediamine; enzymes, e.g. proteolytic, lipolytic and cellulolytic enzymes; perfumes, colouring agents, anti-corrosion agents, fabric damage inhibitors, anti-soil redeposition agents and so on. The particulate detergent composition is obtained by normal processes like spray-drying an aqueous slurry of the constituents, dry-mixing and so on.

In general, the detergent composition may contain from 1-80% of an active detergent material, which may be an anionic, nonionic or cationic synthetic detergent or a soap, or mixtures thereof. When mixtures of synthetic detergents are used, the detergent composition may contain from 2 to 20% by weight of an anionic synthetic detergent, from 1 to 10% by weight of an alkali-metal soap. Furthermore, the composition may contain from 10 to 60% by weight of a water-soluble alkali-metal builder salt, and up to 45% by weight of a bleaching agent. Other enzymes such as proteases, lipases and cellulases can also be added to the compositions of the invention, either as separate granules or incorporated in the amylolytic enzyme-containing granules.

The amount of amylolytic enzyme-containing granules in the detergent composition is generally from 3 to 80% and preferably from 10 to 25% by weight.

The invention will be further illustrated by way of Example.

EXAMPLE I.

A series of amylolytic enzyme-containing granules of the following compositions was prepared:

	Granules					
	A	B	C	D	E	F
granular pentasodium tripolyphosphate	76	76	63	63	76	76
sec. C ₁₁₋₁₅ alcohol, condensed with 7 moles of ethylene oxide	12	12	10	10	12	12
α -amylase (Maxamyl® KA 200) (act. 22.9 IU/mg)	7	7	7	7	—	—
*glycine/Maxamyl® 1200 (ratio 1:5) (act. 24.4 IU/mg)	—	—	—	—	8.5	—
**completely hydrolyzed casein/Maxamyl® KA 200	—	—	—	—	—	8.5
Maxatase® 500,000 (a proteolytic enzyme)	3.5	3.5	3.5	3.5	3.5	3.5
glycine	1.5	—	1.5	—	—	—
sodium sulphate	—	1.5	—	1.5	—	—
water	—	—	15	15	—	—

* This mixture is a freeze-dried mixture, prepared as follows:

37.5 g Maxamyl® 1200 and 7.5 g glycine were dissolved in 100 ml distilled water. 8 drops of polypropylene glycol (MW 2000) were added to prevent foam formation.

This mixture was freeze-dried. The glycine content of the freeze-dried granular composition was 19.7%, the enzyme activity 22.7 IU/mg.

**the same procedure as above was followed with the mixture of 37.5 g Maxamyl® KA 200 and 7.5 g completely hydrolyzed casein. The latter had the following composition:

	%
dry matter	96
NaCl	44
amino acids	52
monosodium glutamate	12
pH (40% solution)	5.0

the activity of the freeze-dried granular mixture was 19.2 IU/mg.

5 The granules A, B, E and F were prepared in the following way: Half of the nonionic surface-active agent was sprayed over the granular sodium tripolyphosphate. Subsequently the enzyme/amino acid or enzyme/Na₂SO₄ mixture was added, and after thorough mixing the other half of the nonionic surface-acting agent was sprayed upon this mixture.

10 The granules C and D were prepared as follows: over the sodium tripolyphosphate an aqueous enzyme/glycine or enzyme/Na₂SO₄ slurry was sprayed. After 20 minutes the non-ionic surface-active agent was sprayed over this mixture.

20 Storage tests were carried out with these granules in a granular detergent composition of the following formulation:

	%	
pentasodium tripolyphosphate	33.6	25
disodium dihydrogen pyrophosphate	2.9	
sec. C ₁₁₋₁₅ alcohol condensed with 7 moles of ethylene oxide	2.0	
sodium sulphate	61.5	

The tests were carried out with the above detergent composition, either conditioned at 30°C and 60% relative humidity or non-conditioned, at 30°C (in closed containers) and at 30°C and 60% relative humidity. The amount of amylolytic enzyme-containing granules, added to the detergent composition was 15%.

The residual amylolytic activity was measured after several periods.

The results were as follows:
Storage condition: 30°C (in closed containers)

40

Residual amylolytic activity (in %) after:

0 days

4 days

8 days

18 days

28 days

Granules

A _o	A _c	B _o	B _c	C _o	C _c	D _o	D _c
100	100	100	100	100	100	100	100
85	96	83	85	107	105	79	88
67	71	56	59	81	79	52	61
44	69	41	46	84	85	47	64
41	65	39	52	87	87	28	65

5 The index o means the granule in the non-conditioned detergent powder, and c in the conditioned detergent powder. From this table it is clear that the granules of the invention, A_o, A_c, C_o and C_c are significantly more stable than the same type of granules without glycine (B_o, B_c, D_o and D_c).

EXAMPLE II.

Example I was repeated, whereby the storage conditions were altered to 30°C and 60% relative humidity. 10

The results were as follows:

Granules

residual amylolytic activity (in %) after:

0 days

4 days

8 days

18 days

28 days

A _o	A _c	B _o	B _c	C _o	C _c	D _o	D _c
100	100	100	100	100	100	100	100
94	108	92	85	110	109	88	87
67	70	63	58	76	90	68	69
71	79	64	64	91	87	60	67
67	75	62	68	92	92	67	70

15 Again the granules of the invention have a significantly better storage stability than the other granules.

EXAMPLE III.

Example I was repeated, using the non-conditioned detergent powder. The products were stored in closed containers at 30°C. 20

The results were as follows:

Granules

residual amylolytic activity (in %) after:

0 days

3 days

14 days

20 days

27 days

A	B	C	D	E	F
100	100	100	100	100	100
93	81	86	77	73	80
47	47	57	44	51	59
36	28	49	37	42	53
34	35	49	30	37	50

25 From these results it appears that the granules of the invention A, C, E and F have storage stabilities superior to those of the other granules.

EXAMPLE IV.

The granules A—E were stored as such at 30°C. For comparison noodles of the following composition were also included in the storage test:

	%
tallow fatty alcohol condensed with	
50 moles of ethylene oxide	70
Maxamyl® KA 200	20
Maxatase 500,000	10

The results were as follows:

residual amylolytic activity (in %) after:	Granules					Noodle
	A	B	C	D	E	
0 days	100	100	100	100	100	100
7 days	107	102	111	95	114	100
14 days	132	126	122	112	123	98
28 days	105	101	104	100	96	91
56 days	65	58	84	76	85	61
84 days	52	42	82	66	99	75
112 days	58	55	84	62	87	67

The granules according to the invention (A, C and E) were significantly more stable than the other granules or than the noodles.

WHAT WE CLAIM IS:—

1. Amylolytic enzyme-containing granules with improved storage stability, said granules containing a stabilizing amount of an amino acid.
2. Granules according to claim 1, wherein the amino acid is an α -amino acid.
3. Granules according to claim 2, wherein the α -amino acid is glycine.
4. Granules according to claim 2, wherein the α -amino acid is the mixture of α -amino acids, obtained by complete hydrolysis of proteins.
5. A detergent composition comprising an organic detergent surfactant, a water-soluble inorganic and/or organic builder salt and amylolytic enzyme-containing granules according to claims 1—4.
6. Process for the preparation of granules according to claims 1—4 comprising attaching an intimate mixture of amylolytic enzymes and an amino acid to a carrier material.
7. Process according to claim 6, wherein the intimate mixture is obtained by precipitation from, absorption from, evaporation of, or

spray drying of, a mother liquor containing amylolytic enzymes and amino acids.

8. Process according to claim 6, wherein the intimate mixture is obtained by freeze-drying an aqueous solution of amylolytic enzymes and amino acids.

9. Process according to claims 6—8, wherein the intimate mixture of amylolytic enzymes and amino acids is attached to a carrier material by means of hydration.

10. Process according to claims 6—8, wherein the intimate mixture of amylolytic enzymes and amino acids is attached to a carrier material by means of conglutination with a nonionic surface active agent.

11. Amylolytic enzyme-containing granules as claimed in claim 1 substantially as herein described.

12. Amylolytic enzyme-containing granules substantially as described in any of the Examples.

13. A process for the preparation of amylolytic enzyme-containing granules as claimed in claim 6 substantially as herein described.

14. A process for the preparation of amylolytic enzyme-containing granules substantially as described in any of the Examples.

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